

US EPA ARCHIVE DOCUMENT

MRID No. 420618-04

DATA EVALUATION RECORD

1. **CHEMICAL:** DPX-Z326-198 (Linuron).
Shaughnessey No. 035506.
2. **TEST MATERIAL:** DPX-Z326-198 (Linuron); N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl-urea; CAS No. 330-55-2; Lot No. 90077284; 98.4% active ingredient.
3. **STUDY TYPE:** Fish Early Life-Stage Chronic Toxicity Test.
Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Pierson, K.B. 1991. Flow-Through, 80 Day Toxicity of DPX-Z326-198 to Embryo and Larval Rainbow Trout, *Oncorhynchus mykiss*. Laboratory Project ID 538-91. Prepared by Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company., Newark, DE. Submitted by Du Pont Agricultural Products, Wilmington, DE. EPA MRID No. 420618-04.
5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M. Rifici*
Date: *4/9/92*
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *4/9/92*
- for* Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA
H.T. Craven
8/26/93
Signature: *John Noles*
Date: *8/26/93*
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requirements for an early life-stage toxicity test using rainbow trout. Fish length was significantly reduced at all exposure levels. Therefore, the MATC of DPX-Z326-198 could not be determined from this test. The NOEC was <0.042 mg/l, the lowest mean measured concentration tested.
8. **RECOMMENDATIONS:** N/A.



9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Rainbow trout (*Oncorhynchus mykiss*) eggs and sperm were purchased from a commercial supplier in Hampton, NH. They were allowed to warm up from 8.4 to 12.7°C, were mixed, and allowed to sit for about 15 minutes. The fertilized eggs were washed with dilution water and transferred to holding tanks (11°C) under dim light (0-4.3 lux).

B. **Test System:** An intermittent-flow proportional diluter was used. Diluter operation was checked daily. The test chambers were 21-l glass aquaria (41 x 20.5 x 26 cm) separated in the center by a glass sheet. The individual compartments each had a volume of 7 l and a solution depth of 18 cm. Two egg incubation cups were suspended in each replicate compartment and oscillated at a rate of one cycle every 79 seconds. The 5.5-cm diameter glass cups had a volume of 212 ml and screened-covered bottoms. The aquaria were placed in a recirculating water bath set to maintain 10 ±2°C. The photoperiod was 16-hour light/8-hour dark with 30 minute dawn and dusk simulations. Light intensity during egg incubation and hatching was 0-4.3 lux and after hatch was 53.8-86.1 lux.

The dilution water was well water obtained on-site. The results of a characterization of the water are given in Table 1 (attached).

Stock solutions were prepared daily in dilution water. A small quantity of undissolved material was usually present on the bottom of the stock container after mixing.

C. **Dosage:** Eighty-day flow-through chronic toxicity test. Based on a rangefinding test, seven nominal concentrations (0.047, 0.094, 0.19, 0.38, 0.75, 1.5, and 3.0 mg/l) and a dilution water control were selected for the test.

D. **Design:** Each test concentration and control consisted of 2 replicate chambers. The test was initiated when 20 embryos (21 hours post-fertilization) were randomly placed in each incubation cup. Two cups were used per

replicate for a total of 80 embryos per concentration.

Egg chambers were inspected daily and infertile, damaged, or fungused embryos were removed. The number of live embryos was recorded daily. After hatching was complete (test day 35), most alevins remained in the egg cups until swim-up was complete (test day 47). The number of fingerlings per replicate was randomly thinned to 15 and they were released into the aquarium. The number of live fingerlings was determined daily. On test day 48 (1 day post swim up), the fish were fed newly-hatched brine shrimp *ad libitum* twice. From days 49 through 59, brine shrimp feedings were increased to 3 times daily. On day 60, frozen adult brine shrimp was offered three times. From day 61 to the end of the test, the fish were fed frozen adult brine shrimp *ad libitum* twice daily. Uneaten food and debris were removed when necessary. Length of surviving trout after 47 (sampled at thinning) and 80 days (test termination) was determined. Wet weight of surviving fish was determined at test termination.

The dissolved oxygen concentration, temperature, and pH were measured in each replicate at test initiation and weekly thereafter. The temperature of a control replicate was also measured continuously using a recording thermometer. The conductivity, hardness, and alkalinity of a dilution water were measured at test initiation and weekly thereafter.

Samples from each replicate were taken at test initiation, on day 6 and weekly thereafter until test termination for quantitative analysis of DPX-Z326-198 by HPLC.

- E. **Statistics:** Length of surviving trout was analyzed using analysis of variance (ANOVA) and Dunnett's test. Shapiro-Wilk's and Bartlett's test were used to evaluate normality and homogeneity of variance. Fish weight data were not normally distributed and therefore were analyzed using the Kruskal-Wallis test. Embryo and larval survival were analyzed using a combination of Fisher's Exact test and the Cochran-Armitage trend test. The LC_{50} values were determined using probit analysis.

12. **REPORTED RESULTS:** Small quantities of undissolved material were usually present on the bottom of the stock container, but were not in the diluter mixing chambers, in the egg cups or test aquaria. The mean measured concentrations were

0.042, 0.11, 0.21, 0.39, 0.79, 1.6, and 3.2 mg/l (Table I, attached).

Embryo survival at day 47 (end of swim up) was significantly affected at 3.2 mg/l. Fingerling survival at days 47 and 80 was affected at concentrations ≥ 0.39 mg/l. The 47-day LC_{50} was 0.46 mg/l (95% C.I. = 0.39-0.57 mg/l). The 80-day LC_{50} was 0.38 mg/l (95% C.I. = 0.25-1.0 mg/l). Fish weight at test termination was significantly lower than the control at concentrations ≥ 0.21 mg/l (Table 31, attached). Fish length at 47 and 80 days in all treatment levels was significantly lower than the control. Toxicant-related behavioral effects were observed at 0.39 mg/l.

All water quality parameters were within expected ranges. The pH of the test solutions ranged from 7.0 to 7.8. Dissolved oxygen concentration ranged from 7.1 to 11.2 mg/l or 63 to 99% of saturation at 10°C (reviewer calculated). The temperature was reported as 9.6-11.5°C during the study. The total alkalinity, hardness, and conductivity of the dilution water was 77-96 mg/l as $CaCO_3$, 66-87 mg/l as $CaCO_3$, and 130-155 μ mhos/cm, respectively, during the exposure.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Fish standard length was the most sensitive parameter. The no-observed-effect concentration (NOEC) value could not be determined for the test because of significant effect on fish length at all treatment levels.

Quality Assurance documentation with audit types and dates was provided in the report. A GLP statement was included indicating adherence to USEPA GLP Regulations for FIFRA (40 CFR 160).

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure: The test procedures were generally in accordance with the SEP and ASTM (1987), except for the following:

The fish were fed up to the end of the test. They should not have been fed during the final 24 hours of the test.

After swim-up, the fish were fed three times daily. The SEP recommends feeding *ad libitum* four times daily.

Only the conductivity, hardness, and alkalinity of the dilution water were measured weekly. ASTM states that

these parameters must be measured in the control, low, medium, and high concentration test solutions weekly.

The light intensity used during the test (53.8-86.1 lux) was lower than that recommended by the SEP (400-800 lux).

Eggs and sperm should be obtained from 3 females and 3 males. The author did not state how many fish were used as the source of the embryos.

- B. **Statistical Analysis:** Using one-way ANOVA and William's test (Toxstat Version 3.3), survival of fry (after 47 days) at concentrations ≥ 0.39 mg/l was significantly lower than the control (see attached printouts 1 and 2). The 47-day LC_{50} (EPA's Toxanal program) was 0.35 mg/l (95% C.I. = 0.29-0.41 mg/l) using the moving average method (see attached printout 2a). Due to 100% mortality at 1.6 and 3.2 mg/l, these levels were excluded from the analysis.

Fry survival at test termination appeared significantly affected at concentrations ≥ 0.39 mg/l. No fry survived to day 80 at concentrations > 0.39 mg/l. Since the data did not pass the homogeneity of variance tests (zero variance at several test levels), non-parametric analyses were performed but proved insensitive. Dunnett's test has historically been very sensitive in situations where heterogeneity exists due to zero variance, and was used to analyze the data (see attached printouts 3 and 4). The results were the same as those of the author. The 80-day LC_{50} was 0.34 mg/l (95% C.I. = 0.26-0.44 mg/l) using the moving average method (see attached printout 4a).

Fry length and weight were analyzed using two-way ANOVA and Bonferroni's test (Crunch Version 3). Fry weight at 0.21 and 0.39 mg/l was significantly lower than the control (see attached printout 6). Length was significantly affected at all exposure levels both at 47 days and 80 days (see attached printouts 5 and 7). The above results were the same as those of the author.

- C. **Discussion/Results:** This study is scientifically sound but does not meet the requirements for an early life-stage toxicity test using rainbow trout. Fish length was significantly reduced at all exposure levels. Therefore, the MATC of DPX-Z326-198 was less than 0.042 mg/l, the lowest mean measured concentration tested.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: No MATC value was generated.
- (3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-01-92.

TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water¹

Parameter	Concentration	Parameter	Concentration
BOD ² , mg/L	<2	Lead ⁵ , mg/L	<0.005
COD, mg/L	13	Magnesium, mg/L	3.5
DOC ³ , mg/L	1.5	MBAS/LAS, mg/L	<0.05
TOC ⁴ , mg/L	<0.5	Mercury, mg/L	<0.00020
Kjeldahl N, mg/L	0.2	Nickel, mg/L	<0.020
Ammonia N, mg/L	<0.05	Nitrite ⁶ , mg/L	<0.1
Turbidity, NTU	<1.0	Nitrate ⁶ , mg/L	1.6
Phenolics, mg/L	<0.050	Phosphate ⁶ , mg/L	<0.1
Color, Co/Pt	<5	Potassium, mg/L	1.8
Solids		Selenium, mg/L	<0.005
total suspended, mg/L	<2	Silver, mg/L	<0.010
Aluminum, mg/L	<0.100	Sodium, mg/L	8.1
Antimony, mg/L	<0.060	Sulfate ⁶ , mg/L	4.8
Arsenic, mg/L	<0.010	Sulfide, mg/L	<0.05
Beryllium, mg/L	<0.001	Thallium, mg/L	<0.01
Boron ⁵ , mg/L	<0.020	Zinc ⁵ , mg/L	<0.020
Cadmium, mg/L	<0.002	Volatile priority	
Calcium, mg/L	25.0	pollutants	ND ⁸
Chloride ⁶ , mg/L	6.3	Acid extractable	
Chromium, mg/L	<0.010	priority pollutants	ND ⁸
Cobalt, mg/L	<0.020	Base/neutral	
Copper, mg/L	<0.010	priority pollutants	ND ⁸
Cyanide, mg/L	<0.025	Pesticides/PCBs	ND ⁸
Iron ⁶ , mg/L	0.053	Organophosphate	
Fluoride ⁶ , mg/L	<0.1	pesticides ⁷ , µg/L	<0.50

¹Date of analyses 17 October 1990 unless indicated otherwise, analyses performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ²Date of analyses 13 December 1990, analyses analysis performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ³Mean value; ⁴Date of analyses 24 July 1990, performed at Du Pont Engineering Test Center, Newark, Delaware; ⁵Below the Published Method Detection Limit (BMDL); ⁶Date of analyses 9 November 1990, analyses performed at Du Pont Chemicals, Jackson Laboratory, Deepwater, New Jersey; ⁷Date of analyses 23 October 1990, analyses performed at Hazleton Laboratories, Inc., Madison, WI; ⁸None detected.

EA-91-068p4

Table I

Measured Concentrations of Active Ingredient in DPX-2326-198 Test Solutions

Nominal Concentration ^a (mg/L)	Measured Test Concentration (mg/L)									
	Day -1 ^b	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Day 41	Day 48	Day 55
H ₂ O Control A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H ₂ O Control B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.047 A	0.047	0.040	0.019 ^{c*}	0.040	0.043	0.038	0.038	0.038	0.038	0.038
0.047 B	---	0.038	0.018 ^{c*}	0.041	0.044	0.045	0.038	0.036	0.036	0.036
0.094 A	0.119	0.119	0.091	0.125	0.127	0.124	0.113	0.117	0.117	0.117
0.094 B	---	0.119	0.093	0.125	0.128	0.122	0.112	0.118	0.118	0.118
0.19 A	---	0.210	0.174	0.228	0.230	0.228	0.207	0.220	0.220	0.220
0.19 B	---	0.212	0.181	0.226	0.230	0.230	0.211	0.220	0.220	0.220
0.38 A	---	0.377	0.332	0.407	0.401	0.414	0.373	0.404	0.404	0.404
0.38 B	---	0.387	0.332	0.409	0.407	0.415	0.374	0.413	0.413	0.413
0.75 A	0.719	0.774	0.688	0.820	0.809	0.840	0.756	0.828	0.828	0.828
0.75 B	---	0.769	0.734	0.831	0.816	0.833	0.752	0.836	0.836	0.836
1.5 A	---	1.59	1.42	1.71	1.68	1.71	1.54	1.67	1.67	1.67
1.5 B	---	1.60	1.41	1.72	1.66	1.72	1.53	1.64	1.64	1.64
3.0 A	2.74	3.15	2.82	3.39	3.32	3.39	3.06	3.28	3.28	3.28
3.0 B	---	3.15	2.85	3.37	3.34	3.40	3.04	3.32	3.32	3.32

^a Contains 98.4% a.i. ^b Original test solutions gave low measured values, due to undissolved test compound in stock. Reported values come from test solutions taken later with a different stock. Values not used to calculate average. ^c Values extrapolated since lowest calibration solution was 0.03 mg/L.

* These values are 50% lower than the true weighted average concentration (True).

A These values are 30% greater than the TWA

C17 0+0
C18 6+13
C19 13+20+27+34+41+
48+55+62+69+74+10
220 76+80
*63

Table I (continued)
Measured Concentrations of Active Ingredient in DPX-2326-198 Test Solutions

068p TWA Conc

Nominal Concentrations ^a (mg/L)	Measured Test Concentration (mg/L)								Average ^b
	Day 48	Day 50	Day 55	Day 62	Day 69	Day 76	Day 80		
H ₂ O Control A	0.00	---	0.00	0.00	0.00	0.00	0.00	0.0	
H ₂ O Control B	0.00	---	0.00	0.00	0.00	0.00	0.00	0.0	
0.047 A	0.039	---	0.042	0.057 ^c	0.050	0.045	0.047	0.041 0.041	
0.047 B	0.039	---	0.048	0.058 ^c	0.052	0.047	0.048	0.042 0.042	
0.094 A	0.109	---	0.119	0.116	0.108	0.099	0.101	0.11 0.113	
0.094 B	0.111	---	0.117	0.117	0.111	0.099	0.101	0.11 0.114	
0.19 A	0.209	---	0.216	0.224	0.209	0.197	0.206	0.21 0.213	
0.19 B	0.211	---	0.216	0.225	0.211	0.197	0.208	0.21 0.214	
0.38 A	0.371	---	0.402	0.399	0.387	0.360	0.372	0.38 0.386	
0.38 B	0.377	---	0.396	0.411	0.390	0.368	0.376	0.39 0.390	
0.75 A	0.751	0.784 ^c	---	---	---	---	---	0.78	
0.75 B	0.758	---	0.788	---	---	---	---	0.79	
1.5 A	1.51	---	---	---	---	---	---	1.6	
1.5 B	1.53	---	---	---	---	---	---	1.6	
3.0 A	3.04	---	---	---	---	---	---	3.2	
3.0 B	3.07	---	---	---	---	---	---	3.2	

^a Contains 98.4% active ingredient.

^b Average concentrations are reported to two significant figures.

^c Submitted due to total mortality of the organisms.

TABLE 31

Summary of Rainbow Trout (*Oncorhynchus mykiss*) Lengths and Weights in the
80 Day Embryo/Larval Test Using DPX-Z326-198 (MR-9118-001; Haskell
Number 18,794)

AT THINNING (DAY 47):

Mean Measured Concentration (mg/L)	Standard Length (cm)			
	A		B	
Control	2.0	+ 0.12 ^a	1.9	+ 0.16 ^a
0.042	1.9	+ 0.072	1.9	+ 0.11
0.11	1.9	+ 0.11	1.9	+ 0.095
0.21	1.9	+ 0.079	1.9	+ 0.060
0.39	1.9	+ 0.075	1.9	+ 0.053
0.79	- ^b	-	-	-
1.6	-	-	-	-
3.2	-	-	-	-

^aStandard deviation.

^bDash denotes no larvae were discarded at thinning.

AT TEST END (80 DAYS):

Mean Measured Concentration (mg/L)	Standard Length (cm)				Wet Weight (g)			
	A		B		A		B	
Control	3.6	+ 0.18 ^c	3.5	+ 0.20 ^c	0.66	+ 0.088 ^c	0.74	+ 0.069 ^c
0.042	3.3	+ 0.25	3.1	+ 0.42	0.64	+ 0.072	0.69	+ 0.21
0.11	3.1	+ 0.32	3.1	+ 0.21	0.63	+ 0.091	0.63	+ 0.099
0.21	2.9	+ 0.37	3.0	+ 0.25	0.47	+ 0.12	0.50	+ 0.089
0.39	2.5	+ 0.21	2.4	+ 0.22	0.23	+ 0.063	0.19	+ 0.068
0.79	- ^d	-	-	-	-	-	-	-
1.6	-	-	-	-	-	-	-	-
3.2	-	-	-	-	-	-	-	-

^cStandard deviation.

^dDash denotes no larvae were alive at test end.

TITLE: 420618-04, linuron, larval survival, day 47

FILE: a:42061804.dtl

TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	0.9500	1.3453
1	control	2	0.9000	1.2490
1	control	3	0.9000	1.2490
1	control	4	0.8000	1.1071
2	0.042	1	0.8000	1.1071
2	0.042	2	0.7500	1.0472
2	0.042	3	0.7000	0.9912
2	0.042	4	0.8000	1.1071
3	0.11	1	0.8500	1.1731
3	0.11	2	0.7500	1.0472
3	0.11	3	0.9000	1.2490
3	0.11	4	0.9000	1.2490
4	0.21	1	0.9500	1.3453
4	0.21	2	0.8000	1.1071
4	0.21	3	0.7500	1.0472
4	0.21	4	0.8000	1.1071
5	0.39	1	0.4500	0.7353
5	0.39	2	0.6000	0.8861
5	0.39	3	0.6500	0.9377
5	0.39	4	0.5500	0.8355
6	0.79	1	0.0500	0.2255
6	0.79	2	0.1000	0.3218
6	0.79	3	0.0500	0.2255
6	0.79	4	0.0000	0.1120

Shapiro Wilks test for normality

Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance

Data PASS homogeneity test at 0.01 level. Continue analysis.

420618-04, linuron, larval survival, day 47

File: a:42061804.dtl

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	0.887	1.238	1.238
2	0.042	4	0.762	1.063	1.131
3	0.11	4	0.850	1.180	1.131
4	0.21	4	0.825	1.152	1.131
5	0.39	4	0.563	0.849	0.849
6	0.79	4	0.050	0.221	0.221

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1.238				
0.042	1.131	1.581		1.73	k= 1, v=18
0.11	1.131	1.581		1.82	k= 2, v=18
0.21	1.131	1.581		1.85	k= 3, v=18
0.39	0.849	5.795	*	1.86	k= 4, v=18
0.79	0.221	15.144	*	1.87	k= 5, v=18

s = 0.095

Note: df used for table values are approximate when v > 20.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

RIFICI LINURON ONCORHYNCHUS MYKISS 03-31-92

47-day EC_{50}

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.2	71	71	100	0
1.6	71	71	100	0
.79	71	67	94.36619	0
.39	71	26	36.6197	0
.21	71	5	7.0423	0
.11	71	3.000001	4.2254	0
.042	71	10	14.0845	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .4492369

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
6	2.141611E-02	← .3475398	.28982 — 0.4141408
.4141408			LMR 4/1/97

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.127973	30.19564	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.457109
95 PERCENT CONFIDENCE LIMITS = -.1524897 AND 5.066708

LC50 = .3593317
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .109302
95 PERCENT CONFIDENCE LIMITS = 0 AND .3056204

TITLE: 420618-04, linuron, fry survival, day 80
 FILE: a:42061804.dt2
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	0.8670	0.8670
1	control	2	0.9330	0.9330
2	0.042	1	0.9330	0.9330
2	0.042	2	0.8000	0.8000
3	0.11	1	0.8000	0.8000
3	0.11	2	0.8670	0.8670
4	0.21	1	0.8670	0.8670
4	0.21	2	0.8670	0.8670
5	0.39	1	0.4000	0.4000
5	0.39	2	0.4000	0.4000

Shapiro Wilks test for normality

Data PASS normality test at P=0.01 level. Continue analysis.

Hartley test for homogeneity of variance

Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.

Additional transformations are useless.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.353	0.088	33.258
Within (Error)	5	0.013	0.003	
Total	9	0.366		

Critical F value = 5.19 (0.05,4,5)

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

420618-04, linuron, fry survival, day 80

File: a:42061804.dt2

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.900	0.900		
2	0.042	0.867	0.867	0.650	
3	0.11	0.834	0.834	1.291	
4	0.21	0.867	0.867	0.641	
5	0.39	0.400	0.400	9.707	*

Dunnett table value = 2.85 (1 Tailed Value, P=0.05, df=5,4)

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.042	2	0.147	16.3	0.034
3	0.11	2	0.147	16.3	0.066
4	0.21	2	0.147	16.3	0.033
5	0.39	2	0.147	16.3	0.500

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

puntent 4a

RIFICI LINURON ONCORHYNCHUS MYKISS 03-31-92

80-day LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.2	27	27	100	0
1.6	27	27	100	0
.79	27	27	100	0
.39	27	15	55.5556	0
.21	30	2	6.666667	0
.11	27	0	0	0
.042	27	1	3.7037	0

THE BINOMIAL TEST SHOWS THAT .21 AND .39 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .3672242

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
6	4.328409E-02	← .3404395	.2612878 - 0.4370032

.4370032

*LMN
4/1/92*

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	11.49676	85.31482	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.074583
95 PERCENT CONFIDENCE LIMITS = -9.741049 AND 17.89022

LC50 = .3501045
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1708084
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Analysis of Variance

Analysis of fish length -- day 80

Date: 03-31-1992

N's, means and standard deviations based on dependent variable: LENGTH

Factors: C R	N	Mean	S.D.
* *	120	3.1200	0.4172
1 * = control	27	3.5519	0.1909
2 * = 0.042	26	3.1846	0.3390
3 * = 0.11	27	3.1296	0.2701
4 * = 0.21	28	2.9286	0.3137
5 * = 0.39	12	2.4333	0.2146
* 1	61	3.1311	0.4197
* 2	59	3.1085	0.4178
1 1	13	3.5692	0.1843
1 2	14	3.5357	0.2023
2 1	14	3.2500	0.2534
2 2	12	3.1083	0.4166
3 1	14	3.1143	0.3207
3 2	13	3.1462	0.2145
4 1	14	2.9000	0.3721
4 2	14	2.9571	0.2533
5 1	6	2.4833	0.2137
5 2	6	2.3833	0.2229

RSD

5.2

5.12

QA OK
LMP 4/1/92

Fmax for testing homogeneity of between subjects variances: 5.11
 Number of variances= 10 df per variance= 10.

Source	df	SS (H)	MSS	F	P
Between Subjects	119	20.7120			
C (CONC)	4	11.8306	2.9577	37.463	0.0000
R (REP)	1	0.0228	0.0228	0.289	0.5920
CR	4	0.1741	0.0435	0.551	0.7005
Subj w Groups	110	8.6844	0.0789		

Post-hoc tests for factor C (CONC)

Level	Mean
1	3.552
2	3.185
3	3.130
4	2.929
5	2.433

Comparison	Bon-ferroni	Dunnett
1 > 2	0.0000	0.0100
1 > 3	0.0000	0.0100
1 > 4	0.0000	0.0100
1 > 5	0.0000	0.0100
2 > 3		N.A.
2 > 4	0.0114	N.A.
2 > 5	0.0000	N.A.
3 > 4	0.0915	N.A.
3 > 5	0.0000	N.A.
4 > 5	0.0000	N.A.

For Dunnett's test only the P-values .05 and .01 are possible
 and only for comparisons with the control mean (level 1).

Analysis of Variance

Fish weight -- day 80

Date: 03-31-1992

* Indicates statistics are collapsed over this factor

Factors: C R	N	Mean	S.D.	RSD
* *	120	0.5768	0.1796	
1 * = <i>control</i>	27	0.7011	0.0884	
2 * = 0.042	26	0.6600	0.1527	
3 * = 0.11	27	0.6270	0.0931	
4 * = 0.21	28	0.4879	0.1031	
5 * = 0.39	12	0.2108	0.0653	
* 1	61	0.5603	0.1579	
* 2	59	0.5937	0.1995	
1 1	13	0.6569	0.0878	13.4
1 2	14	0.7421	0.0690	9.3
2 1	14	0.6357	0.0722	
2 2	12	0.6883	0.2127	
3 1	14	0.6257	0.0910	
3 2	13	0.6285	0.0989	
4 1	14	0.4721	0.1170	
4 2	14	0.5036	0.0885	
5 1	6	0.2283	0.0631	
5 2	6	0.1933	0.0683	

Fmax for testing homogeneity of between subjects variances: 11.37
 Number of variances= 10 df per variance= 10.

Source	df	SS (H)	MSS	F	P
Between Subjects	119	3.8394			
C (CONC)	4	2.4940	0.6235	54.094	0.0000
R (REP)	1	0.0367	0.0367	3.182	0.0772
CR	4	0.0408	0.0102	0.885	0.4792
Subj w Groups	110	1.2679	0.0115		

Post-hoc tests for factor C (CONC)

Level	Mean
1	0.701
2	0.660
3	0.627
4	0.488
5	0.211

Comparison	Bon- ferroni	Dunnett
1 > 2		
1 > 3		
1 > 4	0.0000	0.0100
1 > 5	0.0000	0.0100
2 > 3		N.A.
2 > 4	0.0000	N.A.
2 > 5	0.0000	N.A.
3 > 4	0.0000	N.A.
3 > 5	0.0000	N.A.
4 > 5	0.0000	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance

FISH LENGTH - DAY 47

Date: 03-31-1992

Factors: C R

	N	Mean	S.D.
* *	158	1.9095	0.1133
1 * = control	41	1.9780	0.1509
2 * = 0.042	31	1.8871	0.0922
3 * = 0.11	35	1.8914	0.1011
4 * = 0.21	36	1.8861	0.0723
5 * = 0.39	15	1.8667	0.0617
* 1	78	1.9295	0.1163
* 2	80	1.8900	0.1074
1 1	22	2.0318	0.1249
1 2	19	1.9158	0.1573
2 1	16	1.8875	0.0719
2 2	15	1.8867	0.1125
3 1	14	1.8786	0.1122
3 2	21	1.9000	0.0949
4 1	20	1.9000	0.0795
4 2	16	1.8687	0.0602
5 1	6	1.8833	0.0753
5 2	9	1.8556	0.0527

6.1
8.2OK
LWR 4/1/92

Fmax for testing homogeneity of between subjects variances: 8.91
 Number of variances= 10 df per variance= 13.

Source	df	SS (H)	MSS	F	P
Between Subjects	157	2.0158			
C (CONC)	4	0.2669	0.0667	6.185	0.0001
R (REP)	1	0.0496	0.0496	4.595	0.0337
CR	4	0.1030	0.0258	2.388	0.0533
Subj w Groups	148	1.5963	0.0108		

Post-hoc tests for factor C (CONC)

Level	Mean
1	1.978
2	1.887
3	1.891
4	1.886
5	1.867

Comparison	Bon- ferroni	Dunnett
1 > 2	0.0034	0.0100
1 > 3	0.0041	0.0100
1 > 4	0.0017	0.0100
1 > 5	0.0053	0.0100
2 < 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
3 > 4		N.A.
3 > 5		N.A.
4 > 5		N.A.

For Dunnett's test only the P-values .05 and .01 are possible
 and only for comparisons with the control mean (level 1).

Study/Species/Lab/ MRID # _____ Chemical z a i. _____ Results _____ Reviewer/ Validation Date _____ Status _____

Chronic Fish _____ Concentrations Tested (pp_m) - 0.042, 0.11, 0.21, 0.39, 0.79, 1.6 3.2

Species: Oncorhynchus mykiss MATC - > 0.042 pp_m 3.2 LNR Supplemental 4/1/92

Lab: Haskell Lab. Toxicol. Indust. Medicine, du Pont

MRID # 420618-04 Control Mortality (%) - 90 Solvent Control Mortality (%) - N/A X
Comments: * mean measured concentrations

Chronic Invertebrate _____ Concentrations Tested (pp_m) - _____

Species: _____ MATC - > _____ < _____ pp_m _____

Lab: _____ Effected Parameters - _____

MRID # _____ Control Mortality (%) - _____ Solvent Control Mortality (%) - _____

Comments: _____

Data listing DAY 80 - RAW DATA File: linuron

Date: 03-31-1992

Obs.	CONC	REP	LENGTH	WIEGHT
1	1	1	3.5	0.67
2	1	1	3.6	0.60
3	1	1	3.6	0.62
4	1	1	3.6	0.64
5	1	1	3.6	0.68
6	1	1	3.8	0.69
7	1	1	3.8	0.75
8	1	1	3.3	0.53
9	1	1	3.5	0.71
10	1	1	3.3	0.50
11	1	1	3.7	0.74
12	1	1	3.8	0.81
13	1	1	3.3	0.60
14	1	2	3.5	0.80
15	1	2	3.2	0.76
16	1	2	3.2	0.67
17	1	2	3.8	0.74
18	1	2	3.2	0.65
19	1	2	3.5	0.65
20	1	2	3.7	0.72
21	1	2	3.7	0.91
22	1	2	3.6	0.70
23	1	2	3.7	0.72
24	1	2	3.6	0.80
25	1	2	3.5	0.73
26	1	2	3.7	0.77
27	1	2	3.6	0.77
28	2	1	3.5	0.69
29	2	1	3.3	0.71
30	2	1	2.9	0.61
31	2	1	3.6	0.77
32	2	1	3.6	0.69
33	2	1	3.5	0.67
34	2	1	3.3	0.52
35	2	1	3.1	0.57
36	2	1	3.2	0.66
37	2	1	2.8	0.58
38	2	1	3.1	0.70
39	2	1	3.0	0.56
40	2	1	3.2	0.58
41	2	1	3.4	0.59
42	2	2	3.2	0.72
43	2	2	3.2	0.74
44	2	2	3.2	0.77
45	2	2	3.7	0.79
46	2	2	2.1	0.10
47	2	2	3.3	0.75
48	2	2	3.5	0.94
49	2	2	3.0	0.73
50	2	2	3.3	0.82
51	2	2	2.6	0.48
52	2	2	3.0	0.70
53	2	2	3.2	0.72
54	3	1	3.0	0.57
55	3	1	3.0	0.69

Obs.	CONC	REP	LENGTH	WIEIGHT
56	3	1	3.2	0.67
57	3	1	3.2	0.69
58	3	1	3.0	0.58
59	3	1	3.0	0.51
60	3	1	3.2	0.59
61	3	1	3.4	0.73
62	3	1	3.5	0.40
63	3	1	2.2	0.73
64	3	1	3.1	0.66
65	3	1	3.0	0.68
66	3	1	3.3	0.61
67	3	1	3.5	0.65
68	3	2	2.8	0.36
69	3	2	3.2	0.63
70	3	2	3.2	0.67
71	3	2	3.3	0.76
72	3	2	3.1	0.65
73	3	2	3.2	0.63
74	3	2	3.0	0.53
75	3	2	3.2	0.68
76	3	2	3.6	0.67
77	3	2	3.4	0.72
78	3	2	2.9	0.58
79	3	2	3.0	0.63
80	3	2	3.0	0.66
81	4	1	3.0	0.47
82	4	1	3.0	0.59
83	4	1	2.4	0.33
84	4	1	3.8	0.78
85	4	1	3.1	0.47
86	4	1	3.2	0.53
87	4	1	2.7	0.46
88	4	1	2.9	0.49
89	4	1	2.7	0.37
90	4	1	3.2	0.52
91	4	1	2.4	0.39
92	4	1	2.9	0.42
93	4	1	2.8	0.47
94	4	1	2.5	0.32
95	4	2	3.2	0.63
96	4	2	2.8	0.50
97	4	2	3.1	0.41
98	4	2	3.0	0.58
99	4	2	2.8	0.41
100	4	2	2.9	0.48
101	4	2	2.5	0.45
102	4	2	2.5	0.36
103	4	2	3.0	0.42
104	4	2	3.2	0.58
105	4	2	3.4	0.62
106	4	2	3.0	0.48
107	4	2	2.9	0.52
108	4	2	3.1	0.61
109	5	1	2.3	0.22
110	5	1	2.2	0.14

Obs.	CONC	REP	LENGTH	WIEGHT
111	5	1	2.4	0.17
112	5	1	2.7	0.26
113	5	1	2.6	0.29
114	5	1	2.7	0.29
115	5	2	2.4	0.21
116	5	2	2.2	0.12
117	5	2	2.4	0.13
118	5	2	2.2	0.19
119	5	2	2.8	0.31
120	5	2	2.3	0.20

Data listing DAY 47 - LENGTH DATA

File: linuron2

Date: 03-31-1992

Obs.	CONC	REP	LENGTH
1	1	1	2.1
2	1	1	2.1
3	1	1	1.9
4	1	1	1.9
5	1	1	2.1
6	1	1	2.2
7	1	1	2.1
8	1	1	1.9
9	1	1	2.0
10	1	1	2.2
11	1	1	1.9
12	1	1	2.2
13	1	1	1.9
14	1	1	2.2
15	1	1	1.9
16	1	1	1.9
17	1	1	2.2
18	1	1	1.9
19	1	1	2.0
20	1	1	2.1
21	1	1	2.1
22	1	1	1.9
23	1	2	2.0
24	1	2	1.9
25	1	2	1.5
26	1	2	2.0
27	1	2	2.0
28	1	2	1.9
29	1	2	2.0
30	1	2	1.8
31	1	2	1.8
32	1	2	2.0
33	1	2	1.9
34	1	2	2.0
35	1	2	1.7
36	1	2	2.1
37	1	2	1.7
38	1	2	1.9
39	1	2	2.1
40	1	2	2.1
41	1	2	2.0
42	2	1	1.9
43	2	1	1.9
44	2	1	1.9
45	2	1	2.0
46	2	1	1.9
47	2	1	1.9
48	2	1	1.8
49	2	1	1.8
50	2	1	2.0
51	2	1	1.9
52	2	1	1.8
53	2	1	1.8
54	2	1	2.0
55	2	1	1.9

Obs.	CONC	REP	LENGTH
56	2	1	1.8
57	2	1	1.9
58	2	2	1.9
59	2	2	1.7
60	2	2	1.8
61	2	2	1.9
62	2	2	1.7
63	2	2	2.0
64	2	2	2.0
65	2	2	1.8
66	2	2	1.8
67	2	2	1.9
68	2	2	2.0
69	2	2	1.9
70	2	2	1.9
71	2	2	2.1
72	2	2	1.9
73	3	1	1.9
74	3	1	1.9
75	3	1	1.8
76	3	1	1.8
77	3	1	1.8
78	3	1	1.8
79	3	1	2.0
80	3	1	2.1
81	3	1	1.9
82	3	1	2.0
83	3	1	2.0
84	3	1	1.8
85	3	1	1.8
86	3	1	1.7
87	3	2	2.0
88	3	2	1.7
89	3	2	1.8
90	3	2	1.9
91	3	2	1.9
92	3	2	2.0
93	3	2	2.0
94	3	2	2.0
95	3	2	2.0
96	3	2	2.0
97	3	2	1.8
98	3	2	1.7
99	3	2	1.9
100	3	2	1.9
101	3	2	1.9
102	3	2	2.0
103	3	2	1.8
104	3	2	1.9
105	3	2	1.9
106	3	2	1.9
107	3	2	1.9
108	4	1	2.0
109	4	1	2.0
110	4	1	1.8

Ops.	CONC	REP	LENGTH
111	4	1	1.8
112	4	1	1.9
113	4	1	1.9
114	4	1	1.8
115	4	1	2.0
116	4	1	1.9
117	4	1	2.0
118	4	1	2.0
119	4	1	1.9
120	4	1	1.8
121	4	1	1.9
122	4	1	1.8
123	4	1	1.9
124	4	1	1.9
125	4	1	1.8
126	4	1	2.0
127	4	1	1.9
128	4	2	1.9
129	4	2	1.9
130	4	2	1.8
131	4	2	1.9
132	4	2	1.9
133	4	2	1.8
134	4	2	1.8
135	4	2	1.9
136	4	2	2.0
137	4	2	1.8
138	4	2	1.8
139	4	2	1.9
140	4	2	1.9
141	4	2	1.8
142	4	2	1.9
143	4	2	1.9
144	5	1	2.0
145	5	1	1.9
146	5	1	1.9
147	5	1	1.8
148	5	1	1.9
149	5	1	1.8
150	5	2	1.9
151	5	2	1.9
152	5	2	1.8
153	5	2	1.8
154	5	2	1.9
155	5	2	1.8
156	5	2	1.9
157	5	2	1.9
158	5	2	1.8